Engineering a Hydrogel System for Dental Pulp Regeneration

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Statement of Purpose: The long term prognosis of the tooth is dictated by the health of the soft tissue, the dental pulp. The pulp tissue becomes threatened when the structural integrity of the hard protective layer is compromised due to trauma or tooth decay. This failure provides a pathway for microorganisms to enter the pulp, which ultimately leads to the destruction of the tooth and periapical tissue. With dental caries being one of the most prevalent diseases in the world, this issue is of great importance and concern.[1] The current recommended treatment for pulp disease is a root canal, which involves removal of the remaining dental pulp tissue and sealing it with a biocompatible material. The main concern with this treatment is that it cuts off the nutrient supply to the dentin tissue and therefore kills the dentin tissue. A better treatment may be to restore the dental pulp tissue after the diseased tissue is removed. To this end, the restoration of the physiologic and structural properties of the native dentin-pulp complex is the ultimate goal of this research. The basis of our research is the utilization of biomaterials that mimic the 3D architecture of the natural dental pulp. Hydrogels were chosen due to their similar characteristics to natural pulp tissue. Other advantages offered include the ability to vary properties to make the hydrogel permeable enough to allow for cell infiltration and blood vessel formation, while still providing adhesive motifs for cell adhesion and growth. The proper hydrogel system can be developed suitable for dental pump stem cell attachment, proliferation, and differentiation. The functional differentiated cells can then secrete natural extracellular matrix of the pulp, and eventually dentin, to not only regenerate the pulp, but restore functionality of the tooth.

Methods: Gelatin/Hyaluronan hydrogels were formed using polyethylene glycol diacrylate (PEGDA) as the crosslinker. Varying concentrations of PEGDA was used to finely tune the stiffness of the hydrogel, while keeping the ratio of hyaluronan to gelatin constant. Hydrogels with varying ratios of gelatin and hyaluronan were also created while keeping the stiffness of the hydrogel the same. Hydrogels were created at 4 different ratios of gelatin to hyaluronan (75:25, 50:50, 25:75, and 0:100) and mechanical testing was performed on the hydrogels with different concentrations of PEGDA in order to achieve similar stiffnesses of the hydrogels when comparing just the ratio. Dental pulp stem cells (DPSCs) were then incorporated into these hydrogels to determine which substrate would most facilitate dental pulp cell survival and proliferation. The cells were mixed within the hydrogel in order to ensure that they were incorporated into the hydrogel and did not just attach on the surface of the hydrogel or culture plate. The morphology of the cells was also observed under the confocal microscope. Proliferation and morphology were determined by Click-EdU assay and immunochemistry.

Results: Dental pulp stem cells were able to survive in a majority of the hydrogel compositions. The stiffness showed little effect on the viability of the cells, but greatly affected the morphology. DPSCs in the lower stiffness hydrogels were more spread out and elongated whereas DPSCs in the stiffer hydrogels were round shaped. The ratios of each component in the hydrogels also greatly affected DPSC viability and cell morphology. Hydrogels with a greater concentration of gelatin showed an increase in viability compared to hydrogels of even ratio or less gelatin. The hydrogel determined to provide the optimal environment for DPSC attachment, migration, proliferation, and differentiation consisted of 75% gelatin, 25% hyaluronan and had a concentration of 2.5% PEGDA (~10Pa).

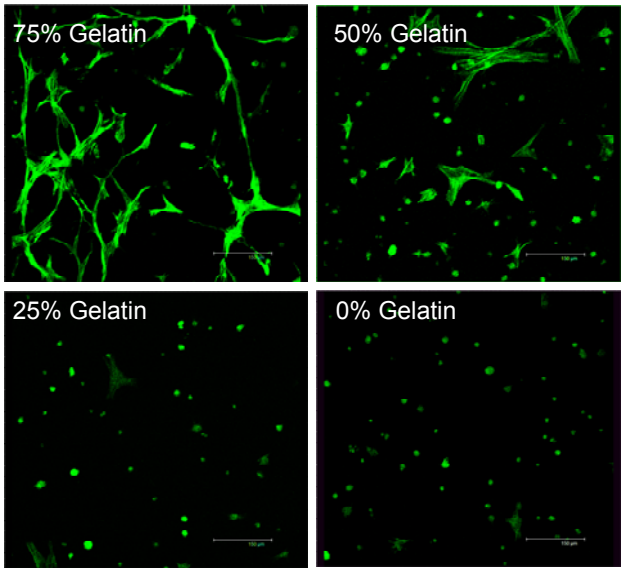

Fig. 1: Morphology study of DPSCs with varying ratios of hyaluronan/gelatin (actin stained in green)

Conclusions: Hydrogels are excellent materials for dental pulp tissue regeneration. Varying the ratios of the hydrogel components and the stiffness of the hydrogel affects cell viability, differentiation, and morphology. The well-spread shape was observed with the stiffness ~ 10Pa. The gelatin component of the hydrogels provides an adhesive motif and therefore promotes viability and spreading of the cells, whereas the hyaluronan provided for a hydrophilic permissive polymer network. The significance of this project is developing a regenerative medicine strategy for the treatment of dental pulp diseases.

References:

1.Wilson,S. et al. Clin Pediatr. 1997;36: 333-7.